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Ethanol Intake During Lactation Impairs Milk Production in Rats and Affects Growth and Metabolism of Suckling Pups

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TAVARES DO CARMO, M. G., C. M. OLLER DO NASCIMENTO, A. MARTIN AND E. HERRERA. *Ethanol intake during lactation impairs milk production in rats and affects growth and metabolism of suckling pups.* ALCOHOL **18**(1) 71–76, 1999.—From parturition, lactating Wistar rats were given 20% alcohol in drinking water and fed a solid diet ad lib (group AL). Pair-fed (PF) and control (C) rats were fed solid diet and given water ad lib (C). All animals were sacrificed on the 12th day of lactation. Ethanol treatment decreased food intake and milk production in lactating rats to a greater level than in PF rats, and a greater reduction in body weight of the AL pups was noted. Brain weight, protein concentration, and DNA content were also lower in pups of AL dams than of PF dams, whereas liver glycogen concentration was higher in the former. Pups from AL dams had higher circulating levels of β-OH-butyrate, triglyceride, and free fatty acids than those from either C or PF dams. Plasma glucose concentration was lower in both PF and AL than in C pups, whereas the AL group had lower plasma protein concentration than any of the other groups. We conclude that maternal alcohol intake during lactation greatly impairs milk production, and although the known increase of lipid content in milk in rats studied under similar conditions allows an enhanced lipidic components in the pups, this adaptation does not allow normal growth and brain development.

Alcohol Lactation Malnutrition Milk production Pup's growth Liver Brain Rat

HUNDREDS of clinical (6,18,20) and experimental studies (7,17,22) have described that alcohol intake during pregnancy causes fetal malformation and growth retardation. The period of lactation, when most of the organs are still developing and brain growth spurt occurs (2), is an important and also vulnerable period, and maternal alcohol exposure during this time could also adversely affect the development of pups.

We have recently found that ethanol treatment in rats throughout lactation produces important metabolic and physiologic disturbances to dams, including alterations in mammary gland composition and its capacity for synthesis of lipids (25). The present study was undertaken to provide information about the possible modifications in several circulating and tissue metabolites and growth rates of suckling rats, in response to maternal ethanol ingestion during lactation. Milk production was also studied in rats at different times during lactation.

METHODS

All procedures involving animals were approved by the Animal Research Committee of the Faculty of Experimental Sciences, Universidad San Pablo-CEU.

Animals and Procedures

Adult female virgin Wistar rats with an initial body weight of 150–200 g, from our own animal quarters, were housed in groups of 4–5 animals per cage and maintained under con-

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trolled conditions of light and dark (12/12 h) and temperature (24 ± 1°C). After breeding, pregnant rats were housed in individual cages and fed Purina chow pellets and water ad lib. The day of parturition was considered day 0 of lactation and litters were adjusted to 8–10 pups per dam. On the first postpartum day dams were divided into three groups (of 12 dams each group): 1) alcohol treated (AL), which received 20% ethanol diluted in drinking water and food ad lib until sacrificed; 2) pair fed (PF), as a nutritional control, that received a solid diet per day and per 100 g of body weight to give an equivalent daily caloric intake as the AL rats. To avoid immediate consumption of the diet, the daily dose was divided in two equal portions for the dark and light periods. 3) Control animals (C) that received a solid diet and tap water ad lib were handled in the same way as the ethanol-treated rats. Daily food and liquid intake and body weight were measured throughout the treatment period (1–12 days). The measure of the pups' weight was taken on days 0, 4, 8, and 12. To account for litter effects, each litter was weighed and the result was divided by the numbers of pups of that litter, then the average of these values was calculated for each group. On day 12 after delivery, pups were decapitated, blood was collected from the neck into dry heparinized beakers, and, simultaneously, a piece of liver and brain was dissected and frozen in liquid nitrogen for further analysis.

Tissue and Circulating Metabolites

After centrifugation of fresh blood, aliquots of plasma were used to measure triglyceride (19), free fatty acids (Colorimetric Kit, Wako, NEFA C test Kit; Wako Chemicals, Germany), and protein (15) concentrations. Other plasma aliquots were deproteinized according to the method of Somogyi (23) to determine glucose (5), glycerol (8), β-hydroxybutyrate (28), and acetoacetate (28) in supernatants.

Brain and liver were excised immediately, weighed, and placed in liquid nitrogen. Aliquots of fresh tissues were homogenized in HCl 2N and analyzed for DNA content following the diphenylamine method (12). Other aliquots were saponified in 3 ml of 30% (w/v) KOH and lipids extracted in petroleum ether (24). Lipid extracts were dried under nitrogen flow and weighed. Protein concentration was measured by the method of Lowry et al (15). Liver glycogen was purified by ethanol extraction and, after alkali digestion, hydrolyzed and glucose determined by the anthrone method (13). Maternal plasma ethanol levels were determined spectrophotometrically using a commercial ethanol diagnostic Kit (Sigma, St. Louis, MO).

Milk Production

Milk production was determined on the basis of fed and fasted pups' weight gain (21) as follows: on days 0, 4, 8, and 12 of lactation each experimental group was divided into two subgroups: i) mothers whose pups were fasting for 24 h, and ii) mothers whose pups were suckling. The fasted and suckled pups of each group were weighed before and after the 24 h. Milk production in each group was estimated according to the following formula:

$$L = Pa_2 - Pa_1 \times (1 - \mathrm{K})$$

where: L = milk production (g/day), Pa_1 = weight of suckled offspring at the beginning of the 24-h period, Pa_2 = weight of suckled offspring at the end of the 24-h period, K = relative loss of weight (average) of fasted offspring.
K is the average of kj ($j = 1, 2 \ldots n$), kj calculated for each pup in a group of n fasted pups as follows:

$$kj = (Pj_1 - Pj_2)/Pj_1$$

where kj = relative loss of weight, Pj_1 = weight of pup j at the beginning of the 24-h period, Pj_2 = weight of pup j at the end of the same period.

Statistical Analysis

Results were expressed as mean ± SEM and statistical comparisons were performed using analysis of variance. When the ANOVA showed that a statistically significant difference existed, Duncan's test was used to determine statistical significance ($p < 0.05$).

RESULTS

Maternal Caloric Intake and Body Weight Changes

Maternal daily caloric intake and weight gain data are summarized in Table 1. The average amount of food intake, alcohol consumption, and weight gain of lactating rats given

TABLE 1

FOOD INTAKE AND BODY WEIGHT OF LACTATING RATS GIVEN ALCOHOL, PAIR FED, OR FED AD LIB

	Food Intake (kcal/g/100 g b.wt.)			Body Weight (g)		
Days	Control	Ethanol	Pair Fed	Control	Ethanol	Pair Fed
0	18.5 ± 1.2	21.7 ± 1.1	20.9 ± 0.8	218.4 ± 6.0	216.5 ± 3.3	223.2 ± 2.9
2	24.3 ± 1.9	19.0 ± 1.2*	18.9 ± 0.9*	220.4 ± 5.1	212.5 ± 2.2	221.2 ± 2.3
4	48.6 ± 6.0	22.8 ± 2.6*	24.9 ± 0.4*	221.6 ± 4.6	197.7 ± 3.6*†	217.7 ± 2.7
6	63.6 ± 5.7	30.1 ± 3.1*	31.9 ± 0.5*	224.5 ± 3.2	189.7 ± 4.0*†	213.7 ± 3.1
8	81.5 ± 6.7	31.5 ± 3.0*	33.5 ± 0.5*	227.0 ± 7.5	184.8 ± 3.1*†	211.6 ± 2.9*
10	73.8 ± 5.4	40.3 ± 5.6*	40.3 ± 0.7*	226.1 ± 5.8	183.6 ± 3.7*†	210.9 ± 3.6*
11	84.2 ± 4.6	45.3 ± 4.0*	43.8 ± 0.6*	228.2 ± 5.9	187.0 ± 3.7*†	207.4 ± 2.6*

Values are the mean ± SEM for 8–12 rats per group.
*Statistical comparison between control and ethanol groups and between control and pair-fed groups: $p < 0.05$.
†Significantly different from pair fed at $p < 0.05$.

alcohol, pair fed, or fed ad lib were similar to our previous findings (25). During lactation, alcohol provided 33% of the caloric intake, which is equivalent to about 12.0 ± 1.2 g of pure ethanol ingested/kg body weight/day. The total caloric intake of the AL and PF groups was approximately 50% of that of the ad lib-fed controls during the lactation period. This was reflected in the significantly lower weight of AL and PF animals, which was even lower in the former than in the latter group despite their equal caloric intake. The mean maternal blood alcohol levels in the AL rats on the 12th day of lactation was 105.3 ± 4.5 mg/dl.

Lactational Performance

Milk yield. Figure 1A shows milk production of the three groups throughout lactation. The milk yield of the AL mothers was greatly compromised in comparison to the other two groups. From the 8th day, C rats greatly increased milk output, whereas in AL rats the quantity of milk produced up to the 12th day of lactation was decreased; PF rats displayed intermediate values, being significantly different than both C and AL rats.

Mean pups weight. The body weight of pups nursed by the three groups of dams is shown in Fig. 1B. The reduced milk production of AL mothers was associated with a significant reduction in corporal weight gain of their pups, and these differences were more marked in the AL group than in the PF group.

Brain and Liver Composition and Circulating Metabolites

Brain. Brain weight was significantly reduced in AL and PF litters when compared with the C group, and values in the AL group were significantly lower than in PF animals (Table 2). On the other hand, when evaluating the brain weight per 100 g of body weight, it was observed that both for AL and PF groups this ratio is higher than for the control group, although this increase was smaller for AL pups.

Brain protein content decreased in AL pups compared to those from both C and PF groups. Regarding the lipids content, no significant differences were found among the three groups studied.

The amount of DNA indirectly expresses the number of cells. As shown in Table 2, pups from PF dams showed a significant increase in brain DNA/g in relation to both C and AL groups, which showed no differences between them. When expressed as DNA per total brain weight AL pups had lower values than those of C or PF pups, suggesting a lower number of brain cells. Total brain DNA content was significantly greater in pups from PF dams than from C rats.

Liver. Both the liver weight and its value corrected by body weight were lower in AL and PF pups than in C (Table 2). Relative liver weight in pups of PF dams was significantly lower than in those of AL dams. The liver of both AL and PF pups had lower protein and glycogen concentration than C pups liver. As expected, values of hepatic glycogen were significantly lower in PF pups than in C and AL pups, the latter having also lower liver glycogen concentration than C. There were no significant differences in liver lipid concentration among the three groups studied. The amount of DNA/g did not differ among the groups but DNA per total liver was significantly lower in the pups of both PF and AL dams, suggesting that the liver of these animals had less cells than C, although cell size was similar.

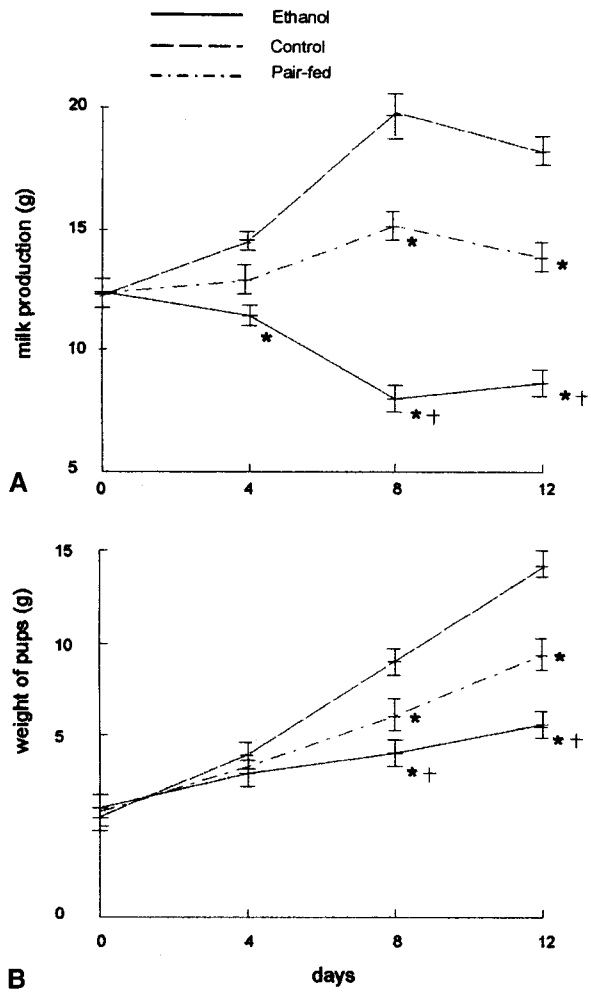

FIG. 1. (A) Milk production and (B) body weights (means ± SEM) of pups from control, pair-fed, and alcohol groups. Significantly different values are shown by: $^{*}p < 0.05$ vs. control group, $^{\dagger}p < 0.05$ vs. pair-fed group.

Plasma nutrients and metabolites. As shown in Table 3, plasma glucose concentration was similarly reduced in AL and PF pups compared to C, with no differences between AL and PF rats. Plasma protein concentration was lower in pups from AL mothers than C, whereas it did not differ between PF and C pups. Plasma triglyceride levels were significantly lower in PF pups than in C and AL, whereas the values of circulating triglycerides in AL pups showed a nonsignificant tendency to rise when compared to the C group. Pups from AL mothers showed a significant increase in plasma levels of free fatty acids when compared to both C and PF groups, whereas no significant differences were detected between these last two groups. Plasma glycerol values were significantly lower in PF than in C and AL groups, and no statistical difference was found between the pups of AL and C mothers. Plasma concentration of β-hydroxybutyrate was significantly higher in PF and AL than in the C group, and the AL group showed values significantly higher than those of the PF group. However, values of acetoacetate did not differ among the three groups.

TABLE 2

EFFECTS OF MATERNAL ETHANOL INTAKE ON LIVER AND BRAIN COMPOSITION OF PUPS ON DAY 12 OF LACTATION

	Control	Pair Fed (PF)	Ethanol (AL)
Brain			
Weight (g)	1.05 ± 0.02	0.91 ± 0.04*	0.78 ± 0.03*†
Relative weight (g/100 g b. wt.)	5.2 ± 0.09	7.5 ± 0.2*	7.0 ± 0.2*†
Protein (mg/g)	105.5 ± 3.7	97.1 ± 2.3	82.1 ± 5.9*†
Lipid (g/100 g)	3.1 ± 0.1	3.4 ±0.1	3.0 ± 0.1
DNA (mg/g)	4.6 ± 0.2	6.2 ± 0.4*	4.4 ± 0.07†
(mg/brain)	4.8 ± 0.2	5.7 ± 0.4*	3.1 ± 0.1*†
Liver			
Weight (g)	0.58 ± 0.02	0.27 ± 0.01*	0.27 ± 0.01*
Relative weight (g/100 g b. wt.)	2.8 ± 0.1	2.2 ± 0.05*	2.5 ± 0.06*†
Protein (mg/g)	222.2 ± 5.8	206.8 ± 3.7*	186.1 ± 4.6*†
Lipid (g/100 g)	3.7 ± 0.1	4.0 ± 0.3	3.8 ± 0.2
Glycogen (mg/100 mg)	3.2 ± 0.3	0.4 ± 0.1*	2.1 ± 0.3*†
DNA (mg/g)	12.9 ± 0.3	13.7 ± 0.5	13.2 ± 1.0
(mg/liver)	7.4 ± 0.5	3.7 ± 0.2*	3.8 ± 0.3*

Values are the mean ± SEM of data from 8–12 litters in each group.
*Significant differences ($p < 0.05$) vs. the control group.
†Significant differences ($p < 0.05$) vs. the pair-fed group.

DISCUSSION

The results obtained in this study show that maternal consumption of ethanol during lactation results in serious modifications in the maternal nutritional status and milk production. The latter was even lower than that of lactating rats receiving the same daily caloric intake, indicating a direct effect of alcohol impairing milk production. Thus, suckling pups of AL mothers are malnourished as reflected in the impaired litter growth. These results agree with those of other investigators using higher levels of ethanol (25%) during pregnancy and lactation (26,27). However, because no PF animals were used in those studies, it could not be established whether the lower body weight of the litter was the result of maternal undernutrition caused by alcohol intake, or a combination of both factors. However, the present results demonstrate that, despite the effect of undernutrition induced by the maternal intake of alcohol, the ethanol itself intensified such effect and even seemed to cause specific effects in the pups.

Our results also show that maternal undernutrition caused by either alcohol intake or pair-fed feeding decreased the weight of both brain and liver in the pups, and that, different to the liver, when corrected by body weight, the brain was heavier in the litter of alcohol and pair-fed mothers.

During the period of rapid brain development, the first 15 days of life in the rat, the brain is extremely vulnerable to possible exogenous influences (3). Under normal conditions, during the period of brain development the weight of the brain increases even more rapidly than body weight. Later, the ratio of brain weight to body weight declines progressively with advancing age (4).

Diaz and Samson (2) found that animals exposed to ethanol from the 4th to the 7th day of birth showed a decrease in brain weight in relation to control animals, without modifying body weight. In the present study we have also observed that pups from AL mothers have lower brain weight than pups from pair-fed mothers, although the ratio of brain weight to

TABLE 3

EFFECTS ON MATERNAL ETHANOL INTAKE ON CIRCULATING METABOLITES OF PUPS ON DAY 12 OF LACTATION

	Control	Pair Fed (PF)	Ethanol (AL)
Glucose (mg/dl)	117.16 ± 4.44	70.45 ± 3.51*	53.98 ± 4.39*
Protein (g/dl)	7.90 ± 0.47	6.94 ± 0.25	5.58 ± 0.64*
Triglyceride (mg/dl)	212.14 ± 41.40	72.57 ± 4.61*	241.50 ± 14.92†
Glycerol (μM)	533.13 ± 21.79	189.89 ± 27.52*	463.16 ± 75.82†
Fatty acids (μM)	710.52 ± 109.76	561.63 ± 68.59	1952.25 ± 167.18*†
β-OH-Butyrate (μM)	1320.50 ± 225.20	5870.37 ± 181.47*	7521.02 ± 361.28*†
Acetoacetate (μM)	374.02 ± 19.84	409.11 ± 36.15	441.42 ± 31.24

Values are the mean ± SEM of data from 9–13 litters in each group. Values that are significantly different from those for control rats are shown by * $p < 0.05$ versus control group and ethanol verus pair-fed group are shown by † $p < 0.05$.
*Significant difference ($p < 0.05$) vs. control group.
†Significant difference ($p < 0.05$) for ethanol vs. pair-fed group.

body weight is greater in the first group. It is possible that this increased ratio reflects the profound reduction in body growth rather than a preservation of the brain, but further studies are necessary to clarify this hypothesis.

These results also show that maternal intake of alcohol during the first 12 days of lactation is more harmful than maternal malnutrition as regards to the decrease in the concentration of protein and DNA (characteristic of hypoplasia) in the brain of the pups, and these alterations might be partially responsible for the lower brain weight observed in the litter of alcoholic mothers. Because it is known that profound malnutrition during suckling has negative effects in brain weight, DNA and protein content (1), the difference in brain composition between the alcohol and the pair-fed group could be a consequence of greater malnutrition in the former, in which maternal milk production was greatly impaired.

The present study also showed that the liver of the litters of both alcohol and pair-fed mothers was smaller than that of controls, and also presented lower concentration of protein, glycogen, and DNA. Although liver protein concentration was lower in pups of alcohol dams than in pair-fed dams, relative liver weight was more impaired in pups of pair-fed dams. The decrease in liver glycogen in pups of alcohol dams was much lower than in pair-fed rats. The differences could be explained by the specific metabolic adaptations taking place during suckling, when the newborn has adapted to the high lipid content of the milk; as previously reported, lipid content is increased in the milk of alcohol-treated rats (26). This interpretation coincides with the higher plasma level of triglycerides, glycerol, free fatty acids, and ketone bodies found in the pups from alcohol dams compared to those of pair-fed and controls.

In the rat, during suckling, adipose tissue is scant (9,10), and therefore the amount of fatty acids and glycerol released into the circulation is not quantitatively relevant. Lipogenesis and triglyceride synthesis are also meager during suckling in the rat (16), and therefore plasma lipids are mainly provided by the milk. An increase in milk lipids in alcohol mothers would therefore justify an abundance of lipids in plasma of their pups. This interpretation also corresponds with the low level of circulating lipidic components seen in pups of pair-fed animals, although ketone bodies is an exception because their level is even higher than those in control pups. Because of the unique presence of lipoprotein lipase activity in the liver of the suckling newborn (14), this organ imports plasma triglycerides to be used as ketogenic substrates. It has been shown that under food-restricted conditions such activity increases (11), allowing an intense ketone body production. Although more direct experiments are required, it is therefore proposed that in pups of the pair-fed group, an enhanced liver lipoprotein lipase activity allows a greater liver ketogenic activity. Therefore, a possibility exists that such enhancement in liver lipoprotein lipase activity also occurs in the pups of alcohol-treated rats, which together with their augmented availability of lipids, justifies the greatly enhanced plasma ketosis. This condition allows to maintain plasma glucose level within a reasonable range and even to maintain in these animals higher liver glycogen stores than in the pair-fed group.

In conclusion, maternal alcohol intake greatly impairs milk production, causing severe undernutrition. This is partially compensated by the enhanced content of lipids in milk, which allows the proper metabolic adaptations to prevent severe hypoglycemia and maintain minimum stores of liver glycogen. However, these adaptations are not enough to protect normal brain development, which is impaired in these animals.

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